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Clinicopathological Variables and p53 Overexpression as a Combined Prognosticator for Hematogenic Recurrence in Colorectal Cancer

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Background and Objectives: Precise evaluation of the prognostic factors for hematogenic recurrence after resection for colorectal cancer is important not only for the prediction of patient outcome but also for the determination of adjuvant therapy. The purpose of the current study was to elucidate the clinical significance of using clinicopathological variables in combination with p53 expression as a prognosticator for hematogenic recurrence.

Methods: One hundred forty-two patients with colorectal cancer were examined. The expression of p53 was determined by immunohistochemical staining.

Results: Eighteen (60%) of the 30 patients who were positive for both p53 overexpression and lymph node metastasis, 13 (41%) of the 32 patients who were positive for p53 and venous invasion, and 13 (39%) of the 33 patients who were positive for p53 and carcinoembryonic antigen (CEA) developed hematogenic recurrence.

Conclusions: The combination of p53 overexpression and lymph node metastasis was an excellent prognostic indicator for hematogenic recurrence in colorectal cancer.

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KEY WORDS: p53 overexpression; prognostic factor; hematogenic recurrence; colorectal cancer

INTRODUCTION

Historically, lymph node metastasis has been recognized as a reliable prognostic factor for colorectal cancer recurrence since the report of Miles in 1920 [1]. Although many clinicopathological determinants besides lymph node metastasis have been indicated, only a few are generally recognized as prognostic factors for colorectal cancer: venous invasion [2], tumor budding [3], DNA ploidy pattern [4], or serum carcinoembryonic antigen (CEA) level [5]. Of late, investigators have reported the clinical significance of some molecular biological variables as prognostic factors for tumor proliferation or metastasis in colorectal cancer: p53 [6], proliferating cell nuclear antigen (PCNA) [7], and nm23 [8]. Overexpression of p53, a promising molecular biological marker, is reported to indicate poor prognosis [9,10].

The prognosis for patients with colorectal cancer is mostly affected by the extent of disease and recurrence after resection of the tumor. Among patients who have similar initial lesions and resections, whether they show hematogenic recurrence is the most influential prognostic

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factor. Precise evaluation of the prognostic factors is important not only for prediction of patient outcome but also for the determination of adjuvant therapy. However, it is rather imprecise to determine patient prognosis using only one indicator. To our knowledge, no other studies have examined the possibility of using combinations of clinicopathological variables with molecular biological variables as prognostic factors in colorectal cancer.

The purpose of the current study was to elucidate the clinical significance of using clinicopathological variables in combination with p53 expression as a prognosticator for hematogenic recurrence after colorectal cancer resection.

MATERIALS AND METHODS

Patients

We reviewed the cases of 142 randomly selected patients with advanced colorectal cancer who were treated in the Department of Surgery, St. Marianna University School of Medicine, Toyoko Hospital, and in the First Department of Surgery, St. Marianna University School of Medicine, from 1986 to 1993. There were 89 patients with colon cancer and 53 with rectal cancer: 72 men and 70 women with a mean age of 63.3 years (range: 36–82 years). Following surgery, patients received adjuvant chemotherapy with oral 5-fluorouracil for 2 years. No patient received radiation therapy. The prognosis for each patient was recorded in the register of the Department of Surgery, St. Marianna University School of Medicine, Toyoko Hospital. Thirty-one patients had hematogenic recurrence after colorectal cancer resection. Hematogenic recurrence occurred in 26 patients with liver metastasis, 6 with pulmonary metastasis, and 1 with cerebral metastasis. (Two types of recurrence occurred in 2 patients at the same time.)

The following clinical and pathologic factors were studied: lymph node metastases, depth of tumor invasion, tumor size, lymphatic invasion, venous invasion, and serum CEA value. Lymph node status was characterized as the presence (n+; 54 patients) or absence (n−; 88 patients) of lymph node metastasis. Depth of tumor invasion was evaluated according to the following criteria: tumor invasion of muscularis propria (mp; 16 patients), tumor invasion of subserosa or serosa (ss/s; 120 patients), or direct tumor invasion of the other organ or structures (si; 6 patients). Tumor size was characterized as less than 5 cm (T−; 56 patients) or more than 5 cm (T+; 86 patients) in diameter. Lymphatic invasion was characterized as the presence (ly+; 118 patients) or absence (ly−; 21 patients) of lymphatic invasion. Venous invasion was characterized as the presence (v+; 60 patients) or absence (v−; 79 patients) of venous invasion. Serum CEA value was characterized as less than 2.5 ng/ml (CEA−; 62 patients) or more than 2.5 ng/ml (CEA+; 61 patients).

Fig. 1. A colorectal cancer stained with CM1. Nuclei of cancer cells show positive for p53.

Immunohistochemistry

For the detection of p53, sections from paraffin-embedded tissue blocks of colorectal cancer obtained from each patient were deparaffinized in xylene and rehydrated through graded alcohols. Antigen retrieval using a pressure cooker (110°C, 2 min) was employed. Endogenous peroxidase activity was blocked by incubation in 0.3% H_2O_2 in methanol. The sections were incubated with a primary anti-p53 antibody (CM1, Novocastra Laboratories Ltd., Newcastle, UK) overnight at room temperature. Following incubation with biotinylated goat anti-rabbit immunoglobulins, the reaction products were visualized with diaminobenzidine tetrahydrochloride. Carcinomas showing more than 20% immunoreactive tumor cells with distinct nuclear staining were considered positive for p53 (p53+; 71 patients) (Fig. 1). Carcinomas showing no distinct nuclear staining or less than 20% immunoreactive tumor cells with distinct nuclear staining were considered negative for p53 (p53−; 71 patients).

TABLE I. Hematogenic Recurrence Rates According to Clinicopathological Variables*

	Recurrence positive	Recurrence negative	*P*
Lymph node metastases			
n−	6 (7%)	82 (93%)	
n+	25 (46%)	29 (54%)	<0.01
Depth of invasion			
mp	1 (6%)	15 (94%)	(NS)
ss/s	28 (23%)	92 (77%)	
si	2 (33%)	4 (67%)	
Tumor size (cm)			
<5	15 (28%)	42 (72%)	(NS)
>5	27 (34%)	69 (66%)	
Lymphatic invasion			
ly−	2 (10%)	19 (90%)	(NS)
ly+	39 (32%)	84 (68%)	
Venous invasion			
v−	16 (19%)	64 (81%)	
v+	26 (46%)	35 (54%)	<0.01
CEA			
−	9 (17%)	53 (83%)	
+	27 (44%)	34 (56%)	<0.01

*mp = muscularis propria; ss = subserosa; s = serosa; si = direct tumor invasion of other organs or structures; CEA− = <2.5 ng/ml; CEA+ = >2.5 ng/ml; NS = not significant.

Statistical Analysis

The independent variables were analyzed by univariate analysis and these variables were included in the following multivariate analysis for evaluation using logistic model. These statistical evaluations were performed using the SD-BASE II statistical software package (MPC, Tokyo, Japan). Correlations between the combinations of clinicopathological variables with p53 expression and hematogenic recurrence rate were analyzed. The χ^2 test was used for statistical analysis. $P < 0.05$ was considered significant.

RESULTS

Twenty of 71 (28%) patients who were positive for p53 and 11 of 71 (15%) who were negative for p53 developed hematogenic recurrences; there was no significant difference ($P = 0.103$ in hematogenic recurrence rate between these two groups. Univariate analysis revealed that lymph node metastases, venous invasion, and serum CEA value were significant prognostic factors ($P = 0.000$, $P = 0.007$, and $P = 0.001$) for hematogenic recurrence. The hematogenic recurrence rate was significantly higher in patients with n+, v+, and CEA+ than those with n−, v−, and CEA− (Table I). The status of lymph node metastases was the most significant variable ($P = 0.000$) in the multivariate analysis (Table II).

Eighteen (60%) of the 30 p53+/n+ patients developed hematogenic recurrence, which was significantly higher ($P = 0.000$) than the other p53/n groups. Thirteen (72%) of the above 18 patients developed hematogenic recurrence within 2 years of the initial surgery (8 in the first year and 5 in the second year). Five more patients developed it later than 2 years. Thirteen (41%) of the 32 p53+/v+ patients developed hematogenic recurrence, which was significantly higher ($P = 0.018$) than the other p53/v groups. Eight (62%) of the above 13 patients developed hematogenic recurrence within 2 years of the initial surgery (5 in the first year and 3 in the second year). Five more patients developed it later than 2 years. Thirteen (39%) of the 33 p53+/CEA+ patients developed hematogenic recurrence, again significantly higher ($P = 0.009$) than the other p53/CEA groups. Ten (77%) of the above 13 patients developed hematogenic recurrence within 2 years of the initial surgery (7 in the first year and 3 in the second year). Three more patients developed it later than 2 years.

There were no significant differences regarding depth of invasion ($P = 0.067$), tumor size ($P = 0.069$), or lymphatic invasion ($P = 0.082$) in combination with p53 (Table IV).

DISCUSSION

The most frequent type of recurrence after surgery for colorectal cancer is hematogenic recurrence, which comprises mostly liver metastasis and pulmonary metastasis. Pre- or postoperative treatment to inhibit hematogenic recurrence is important for improving the prognosis of patients with colorectal cancer. Early diagnosis followed by suitable treatments for recurrence are also indispensable. There are many ways to detect hematogenic recurrence: computed tomography (CT), ultrasound (US), magnetic resonance imaging (MRI), or serum CEA value. However, it is unrealistic to perform frequently all these examinations on all patients within a short interval so as to enable early detection of hematogenic recurrence. Thus, to detect hematogenic recurrence at an early stage, systemic surveillance for high risk patients is not only effective but also economical.

Investigators have reported venous invasion [2] or lymph node metastasis [11] as prognostic clinicopathological variables for hematogenic recurrence. Of late, p53, a suppressor gene, has been widely accepted as a prognosticator for hematogenic recurrence [9,10]. However, these variables are not specific, and there are too many patients at high risk for hematogenic recurrence when it is determined by using only one indicator. To our knowledge, no other studies have referred to the possibility of using combinations of clinicopathological variables with p53 as a prognostic factors in colorectal cancer.

In this study, multivariate analysis revealed that lymph node metastasis was the most influential factor for hematogenic recurrence. To evaluate the prognostic factors

TABLE II. Multivariate Analysis of Prognostic Factors for Hematogenic Recurrence

Factors	Presumptive value	Standard error	Normal deviation	*P*
Lymph node metastases	2.199	0.552	3.984	0.000
Serum CEA value	1.704	0.544	3.123	0.002
Venous invasion	0.725	0.538	1.348	0.180 (NS)[a]
Tumor size	0.867	0.753	1.151	0.252 (NS)
Depth of invasion	1.087	1.123	0.967	0.335 (NS)
Lymphatic invasion	0.335	0.885	0.379	0.705 (NS)

[a]NS = not significant.

TABLE III. Combination of Prognostic Factors Showing Significant Correlations With Hematogenic Recurrence Rates

Combination	Recurrence positive	Recurrence negative	*P*
p53 + lymph node metastasis			
p53−/n−	4 (9%)	43 (91%)	
p53−/n+	7 (29%)	17 (71%)	
p53+/n−	2 (5%)	39 (95%)	
p53+/n+	18 (60%)	12 (40%)	<0.01
p53 + venous invasion			
p53−/v−	4 (10%)	36 (90%)	
p53−/v+	7 (25%)	21 (75%)	
p53+/v−	7 (18%)	32 (82%)	
p53+/v+	13 (41%)	19 (59%)	<0.05
p53 + CEA			
p53−/CEA−	2 (6%)	32 (94%)	
p53−/CEA+	8 (29%)	20 (71%)	
p53+/CEA−	5 (18%)	23 (82%)	
p53+/CEA+	13 (39%)	20 (61%)	<0.01

TABLE IV. Combination of Prognostic Factors Showing No Significant (NS) Correlations With Hematogenic Recurrence Rates

Combination	Recurrence positive	Recurrence negative	*P*
p53 + depth of invasion			
p53−/mp	0	8 (100%)	(NS)
p53−/ss/s	11 (18%)	49 (82%)	
p53−/si	0	3 (100%)	
p53+/mp	1 (13%)	7 (87%)	
p53+/ss/s	17 (28%)	43 (72%)	
p53+/si	2 (67%)	1 (33%)	
p53 + tumor size[a]			
p53−/T−	4 (17%)	19 (83%)	(NS)
p53−/T+	7 (15%)	41 (85%)	
p53+/T−	6 (18%)	27 (82%)	
p53+/T+	14 (37%)	24 (63%)	
p53 + lymphatic invasion			
p53−/ly−	2 (14%)	12 (86%)	(NS)
p53−/ly+	9 (17%)	45 (83%)	
p53+/ly−	0	7 (100%)	
p53+/ly+	20 (31%)	44 (69%)	

[a]T− = <5 cm in diameter; T+ = >5 cm in diameter.

for hematogenic recurrence more precisely, the significance of the clinicopathological variables in combination with p53 was studied. The combinations of p53 overexpression with lymph node metastasis, venous invasion, or elevated serum CEA were influential factors for hematogenic recurrence; patients with p53+/n+, p53+/v+, or p53+/CEA+ were evaluated as at high risk for hematogenic recurrence. Among these prognostic factors, p53+/n+ indicated the worst prognosis because 60% (18/30) of patients with p53+/n+ developed hematogenic recurrence. Only patients with p53+/n+ showed higher recurrence rate than patients with positive lymph node metastases (46%, 25/54) in univariate analysis.

The first step of hematogenic metastasis is the invasion of cancer cells into the microvessels. The mutation of p53 is considered to induce angiogenesis [12], which serves the enhancement of cancer cell invasion. A high level of venous invasion indicates the invasion of cancer cells into peripheral microvessels resulting in hematogenic recurrence. Thus, hematogenic recurrence developed at a high rate in patients who were positive for p53 and showed venous invasion.

Lymph node metastasis occurs through the invasion of cancer cells into lymph vessels. The mechanism of hematogenic metastasis is different from that of lymph node metastasis; however, hematogenic recurrence develops more frequently in patients who are positive for lymph node metastasis than in those who are negative for it. It is unknown whether the mechanism of cancer cell invasion into the lymph vessels is the same as that into the microvessels. However, cancer cells invading lymph vessels might be likely to invade peripheral microvessels. Tomoda and Kakeji [13] reported a close correlation between the overexpression of p53 and lymph node metastasis, however, they did not discuss a correlation of p53 and lymph node metastasis with hematogenic occurrence. Thus, the high frequency of hematogenic recurrence in patients who are positive for p53 overexpression and lymph node metastasis in combination is suspected to be related to the angiogenesis induced by p53 and the high metastatic potency indicated by lymph node metastasis.

The reason for correlation between p53 overexpression and CEA elevation is unclear. The high frequency of hematogenic recurrence in patients who were positive for p53 and had a high level of CEA might only be a reflection of the two independent prognostic factors, each hav-

ing significance, which is heightened by placing them in a combined status as a single factor.

Hematogenic recurrence developed within 2 years of the initial surgery in 72% (13/18), 62% (8/13), 77% (10/13) of the combined p53+/n+, p53+/v+, or p53+/CEA+ groups, respectively. Generally, 70% of patients who developed hematogenic recurrence are diagnosed within 2 years of the initial surgery [14]. An acceptable prognosis can be obtained in patients who are detected with hematogenic recurrence at an early stage and undergo resection for the recurrent tumor: e.g., the 5-year survival rates for patients with hepatic resection are up to 30–40% [15,16] and those with lung resection are up to 24–40.5% [17,18], respectively. Thus, careful surveillance for hematogenic recurrence during the first 2 years following initial surgery is quite important.

The combination of p53 and clinicopathological variables is an excellent prognostic factor for hematogenic recurrence; p53+/n+, p53+/v+, or p53+/CEA+ indicates a high potential for hematogenic recurrence, and p53+/n+ is the worst prognosticator. Thus, intensive adjuvant therapy and careful surveillance should be performed for patients with these prognosticators.

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REFERENCES

1. Miles WE: Discussion on the surgical treatment of cancer of the rectum. Br Med J 1920;2:730–742.
2. Talbot IC, Ritchie S, Leighton MH, et al.: Spread of rectal cancer within veins. Histologic features and clinical significance. Am J Surg 1981;141:15–17.
3. Hase K, Shatney C, Johnson D, et al.: Prognostic value of tumor ''budding'' in patients with colorectal cancer. Dis Colon Rectum 1993;36:627–635.
4. Witzig TE, Loprinzi CL, Gonchoroff NJ, et al.: DNA ploidy and cell kinetic measurements as predictors of recurrence and survival in stages B2 and C colorectal adenocarcinoma. Cancer 1991;68:879–888.
5. Blake KE, Dalbow MH, Concannon JP, et al.: Clinical significance of the preoperative plasma carcinoembryonic antigen (CEA) level in patients with carcinoma of the large bowel. Dis Colon Rectum 1982;25:24–32.
6. Hamelin R, Laurent-Puig P, Olschwang S, et al.: Association of p53 mutations with short survival in colorectal cancer. Gastroenterology 1994;106:42–48.
7. Mayer A, Takimoto M, Fritz E, et al.: The prognostic significance of proliferating cell nuclear antigen, epidermal growth factor receptor, and mdr gene expression in colorectal cancer. Cancer 1993;71:2454–2460.
8. Barnes R, Masood S, Barker E, et al.: Low nm23 protein expression in infiltrating ductal breast carcinomas correlates with reduced patient survival. Am J Pathol 1991;139:245–250.
9. Yamaguchi A, Kurosaka Y, Fushida S, et al.: Expression of p53 protein in colorectal cancer and its relationship to short-term prognosis. Cancer 1992;70:2778–2784.
10. Goh HS, Yao J, Smith DR: p53 point mutation and survival in colorectal cancer patients. Cancer Res 1995;55:5217–5221.
11. Yamamura T, Matsuzaki H, Matsuoka H, et al.: Multivariate analysis on prognostic factors for recurrence after resection of colorectal cancer. J Jpn Soc Coloproctol 1997;50:130–135.
12. Kieser A, Weich HA, Brandner G, et al.: Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. Oncogene 1994;9:963–969.
13. Tomoda H, Kakeji Y: Immunohistochemical analysis of p53 in colorectal cancer regarding clinicopathological correlation and prognostic significance. J Surg Oncol 1995;58:125–128.
14. Yamamura T, Seo K, Adachi Y, et al.: Treatments of metachronous liver metastases from colorectal cancer. J Jpn Surg Assoc 1991;52:741–747.
15. Hughes KS, Simon R, Songhorabodi S, et al.: Resection of the liver for colorectal carcinoma metastases: A multi-institutional study of patterns of recurrence. Surgery 1986;100:278–284.
16. Butler J, Attiyeh FF, Daly JM: Hepatic resection for metastases of the colon and rectum. Surg Gynecol Obstet 1986;162:109–113.
17. Okumura S, Kondo H, Tsuboi M, et al.: Pulmonary resection for metastatic colorectal cancer: Experiences with 159 patients. J Thorac Cardiovasc Surg 1996;112:867–874.
18. Girard P, Ducreux M, Baldeyrou P, et al.: Surgery for lung metastases from colorectal cancer: Analysis of prognostic factors. J Clin Oncol 1996;14:2047–2053.